In re Application of: GEPSTEIN et al
Serial No.: 10/759734
Filed: January 29, 2004
Office Action Mailing Date: October 19, 2006

Examiner: Singh, Anoop Kumar
Group Art Unit: 1632
Attorney Docket: 27395

REMARKS

Reconsideration of the above-identified Application in view of the amendments above and the remarks following is respectfully requested.

Claims 1-195 are in this Application. Claims 1-175, 182-185 and 190-193 have been withdrawn from consideration. Claims 176-181, 186-189 and 194-195 have been rejected under 35 U.S.C. § 112 second paragraph. Claims 176-181, 186-189 and 194-195 have been rejected under 35 U.S.C. § 102 (a). Claims 176-181, 186-189 and 194-195 have been rejected under 35 U.S.C. § 103 (a). Claims 187-195 have now been canceled herwith. Claims 176 and 178-181 have been amended herewith. New claims 196-199 have been added herewith.

Amendments To The Specification

The Examiner has noted the improper use of the legal language "said" in the abstract of the instant application. Applicants have amended the abstract and omitted the above mentioned language.

Amendments To The Claims

Claim Objections

Claims 179-181 were objected to because they are recited to be dependent on a method claim 178, while claim 178 is a composition claim.

Applicant has amended claims 179-181 to be directed at compositions rather than methods, thereby rendering moot Examiner's objections in this case.

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35 U.S.C. § 112 second paragraph Rejections

The Examiner has rejected claims 176-181, 186-189 and 194-195 under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

Specifically, the Examiner states that claims 176-181, 186-189 and 194-195 are vague and indefinite because they recite the term "substantial proliferation", which is subject to variable interpretation depending on the Artisan.

The Examiner further states that the term "substantial" is not defined in the claim, nor is it elaborated in the specification so that one of the ordinary skill in the art could be reasonably appraised of the scope of the invention. The Examiner's rejection is respectfully traversed.

The substantial proliferation of the cells is defined and exemplified in Page 37 lines 14-20 of the instant application as follows:

"The cells and tissues of the present invention also display substantial proliferative qualities. *Preferably, such proliferation is characterized by a proliferation index of at least about 10 %, [...] yet still more preferably about 50 % and most preferably at least about 60 %."* (Emphasis Added)

The proliferative qualities of the cells of the present invention are also presented in Example 4 (see Page 79 lines 15-20), in which the cells and tissues of the present invention displayed a proliferative index of about 60 %, as determined by the expression of Ki-67, a marker of cell cycle activity.

Notwithstanding the above and in order to expedite prosecution in this case, Applicants have elected to remove the word "substantial" from claim 176 and to add new claim 196, which further defines the proliferation characteristics of the culture as claimed.

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The Examiner further states that it is an inherent property of all cells that they proliferate from a single cell for at least 1 day. The Examiner's rejection is respectfully traversed.

Applicants wish to point out that contrary to Examiner's assertion proliferation is not a property which is shared by all cells. In fact, it is well established that terminally differentiated cells do not proliferate at all. For example, Mammalian cardiomyocytes irreversibly withdraw from the cell cycle soon after birth and lose the cell proliferative activity (Olson and Schneider, Genes and Development 17:1937-1956, 2003, attached). In fact, functional transplantation or engraftment of adult cardiomyocytes into affected cardiac tissues within dead or dysfunctional cardiac tissues is impossible to date, due to the inability of adult cardiomyocytes to proliferate, and hence to efficiently colonize and regenerate, dead or damaged cardiac tissue. Conversly, cells of the present invention exhibit a proliferative capacity from as early as day 1 in culture till day 35.

The Examiner further rejected claim 176 as reciting "predominantly" displaying which is not clearly explained. The Examiner's rejection is respectfully traversed.

In order to expedite prosecution of this case, Applicants have elected to remove the term "predominantly" from claim 176, thereby rendering moot Examiner's rejection in this case.

The Examiner further states that the ranges of proliferation and cardiac phenotype are inconsistent and confusing in claim 176. The Examiner's rejection is respectfully traversed.

In order to expedite prosecution in this case, Applicants have elected to rephrase the claim to render moot Examiner's rejection in this case.

The Examiner further states that it is unclear whether cardiac phenotype as recited in claims 176 and 188 are with respect to any disease or experimental condition. The Examiner's rejection is respectfully traversed. Claims 188 has now been cancelled.

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The present invention relates to the formation of proliferative cardiac cells and tissues which exhibit a cardiac phenotype. It would be apparent to the skilled artisan that the cardiac phenotype is a phenotype or a combination of phenotypes which is unique to the cardiac tissue and is not shared by other tissues. The cardiac specific phenotype is well established in any aspect including contractility, morphology, gene expression and the like under healthy, diseased or any developmental condition. This includes cardiac lineage differentiation and specific responses to treatments such as pharachological and electrical treatments, as described in Examples 1 – 4 starting on page 49 of the instant application. It would further be clear to the skilled artisan that such a phenotype can be determined under various test conditions pending on the assay used, each of these conditions is selected suitable for unequivocally determining a cardiac phenotype. Thus, for example, mechanical contraction is determined visually, as described in page 30 lines 29-33, and in Example 1, page 50 lines 19-24, using an optical microscope or microscopic based detection of motion. A cardiac specific structure is determined as described in page 31 lines 1-29, and in Example 1, pages 51-52, using light microscopy, fluorescence affinity labeling and fluorescence microscopy, or electron microscopy. A cardiac specific protein is determined as described in page 31 line 30 to page 32 line 4, using Fluorescence affinity labeling and fluorescence microscopy. A cardiac specific RNA is determined, as described in page 32 line 5-14, and in Example 1, page 55 line 25 to page 56 line 4) using RT-PCR based methods. Cardiac specific changes in the intracellular concentration of a physiological ion is determined, as described in page 32 lines 14-20, and in Example 1, page 53 lines 7-21, using fluorescent ion binding based assays. Cardiac specific electrical activity is determined as described in page 32 line 21 to page 33 line 8, and in Example 2, page 61, line 22 to page 62 line 17, using a multielectrode array, and preferably growing the cells of the present invention directly on the multielectrode array to generate for example electrical activity maps, depicting electrical activity as a function of local activation time at each electrode, which can be used to depict conduction velocity and conduction directionality of propagative electrical activity,

preferably in the form of conduction velocity vectors, of electrical activity propagation over an area of the microelectrode array, as is further illustrated, for example in Figure 9 (page 18 line 26 to page 19 line 5). Cardiac specific electrical activity consists of spontaneous electrical activity, rhythmic electrical activity and synchronized / propagative electrical activity. Thus, for example, spontaneous electrical activity is visually shown for Figure 16b, showing the activation origin (red) in the rat tissue propagating to the rest of the co-culture. Cardiac specific changes in the intracellular concentration of a physiological ion is determined as described in Figures 6a-b (page 18 lines 11-15 by determining the changes of intracellular concentrations of transients. Cardiomyogenesis is determined as suggested in Example 4 (page 79 lines 28-29) by determining cardiomyogenesis specific gene expression profiles, as described above. Pharmacological analysis of conduction was determined as described in page 62 line 18 to page 63 line 5, by evaluating the effects of drugs on conduction through the examination of possible changes in the culture's global velocity (measured as the distance between earliest and latest activation divided by the total microelectrode array activation time), in the mean magnitude of the local velocity vector, and in the maximal absolute value of the first time derivative of the extracellular signal..

The Examiner further states that the term "slow conduction" in claims 187 and 195 is a relative term, which renders the claim indefinite. The Examiner states that the term "slow" is not defined in the claim, nor is it elaborated in the specification so that one of an ordinary skill in the art could be reasonably appraised of the scope of the invention. Claims 187 and 195 have now been canceled.

In view of the above arguments and claim amendments, Applicants believe to have overcome the 35 U.S.C. § 112, second paragraph, rejections.

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35 U.S.C. § 102 Rejections

The Examiner has rejected claims 176-181, 186-189 and 194-195 under 35 U.S.C. 102(a) as being anticipated by Kehat et al., (Circulation, Supplement II Vol. 102 NO18, October 31, 2000 abstract IDS). The Examiner's rejection is respectfully traversed. Applicants respectfully request the withdrawal of this rejection on the grounds that the Kehat publication is not prior art relative to the instant application.

As shown in the attached declarations of Gepstein, Lior; Kehat, Izhak; Joseph, Itskovitz-Eldor; and Amit, Michal; the Kehat et al. publication is Applicants own work which was published within a year of the filing of the instant application, thus rendering moot Examiner's rejections in this case.

The Examiner further rejected claims 176-181, 186-189 and 194-195 under 35 U.S.C. 102(a) as being anticipated by Itskovits-Eldor et al., (Mol Med. 2000 Feb;6(2):88-95, IDS). Claim 176 has now been amended. Claims 188-195 have now been cancelled.

Specifically, the Examiner states that Itskovits-Eldor et al. teach an in vitro culture of human cells of cardio specific lineage obtained from human ES cells that shows the cardiac specific synchronous rhythmic activity. The Examiner's rejection is respectfully traversed.

The present invention relates to the formation of cardiac cells and tissues by in vitro culture of differentiable cells which aggregate to robustly form a ***plurality of*** embryoid bodies that exhibit cardiac gene expression and/or activity.

Attempts of utilizing suspension culture of cystic human embryoid bodies have up to the present invention, been inefficient, have not demonstrated a satisfactory range of cardiac specific structure and function, have not provided isolated human cardiac cells and tissues and have not demonstrated long term cardiac functionality in-vitro. Indeed, while an attempt to produce cells exhibiting cardiac functionality was made by Itskovits-Eldor et al, they have managed to produce merely one EB which possesses cardiac specific synchronous rhythmic activity. This specific EB is shown in Figure 4 titled "cardiac muscle differentiation depicted in *a* pulsing embryoid body"

(page 93), where only one EB is shown to exhibit rhythmic contractions. Indeed as declared by Dr. Michal Amit a co-author in the Itskovitz-Eldor reference only one EB having a cardiac phenotype was obtained in these studies and that one is shown in Figure 4.

Conversely, by culturing human ES cells according to the teachings of the present invention, a plurality of contracting EBs were established. As shown in Figure 2, of the instant application, 8 % of the EB produced in the culture exhibited contracting areas.

Notwithstanding the above and in order to distinguish the claimed invention from the art of Itskovitz-eldor et al., Applicants have elected to amend claim 176 to read over an in vitro culture which comprises a plurality of embryoid bodies which exhibit cardiac phenotype. Support for a plurality of embryoid bodies can be found in page 54 line 8, wherein contracting areas appeared in 153 (8.1 %) embryoid bodies cultured under the teachings of the present invention.

The Examiner further rejects claims 176-181, 186-189 and 194-195 under 35 U.S.C. 102(a) as being anticipated by Xu et al. (US 2005/0164382A1). The Examiner states that Xu et al discloses human cells of the cardiomyocyte lineage which are differentiated into cells showing morphologic markers characteristic of cardiomyocytes and spontaneous periodic contraction. The Examiner's rejection is respectfully traversed.

Applicants respectfully request the withdrawal of this rejection on the grounds that the attached Declaration under 37 CFR 1.132 of Applicants proves that Applicants conceived and reduced to practice the invention (in the Form of Kehat et al. Circulation, Supplement II Vol. 102 NO18, October 31, 2000 abstract IDS) prior to the effective date of Xu et al.(effective filing date 7/12/2001).

In view of the above amendments and remarks Applicants believe to have overcome the 35 U.S.C. § 102 rejections

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35 U.S.C. § 103 Rejections

The Examiner has rejected claims 176-181, 186-189 and 194-195 under 35 U.S.C. 103(a) as being unpatentable over Itkovitz-Eldor et al (Mol Med. 2000 Feb;6(2):88-95,IDS) and Igelmund et al (Pfulers Arch. 1999 Apr;437(5): 669-79).

The Examiner states that since Itskovitz-Eldor taught an in vitro culture of human cells of cardio specific lineage obtained from human ES cells that shows the cardiac specific synchronous rhythmic activity, the cardio specific lineage of human cell disclosed by Iskovich-Eldor and those embraced by the instant claims appear to be structurally same, therefore proliferation potential and cardiac phenotype of these cells will be inherent property of the cells.

The Examiner further states that Igelmund et al teach a method to investigate the spontaneous electrical activity of cardiomyocyte clusters in EBs of small groups of cells and of single cardiomyocytes. Igelmund et al teach single EBs plated for multiple recording from several locations of individual EBs using an electrode matrix for recording population action potentials from the beating areas of EBs to determine the electrical interaction beween cardiomyocytes and beating activity. Igelmund et al conclude that this method of field potential recordings from clusters of ES derived cardiomyocytes with EBs provide a useful tool for studying in vitro chronotropy and action potential propagation. However, Igelmund et al do not explicitly teach recording action potential of human cells. According to the examiner, in view of the teaching of Igelemund et al and Iskovitz-Eldor, it would have been obvious for one of ordinary skill in the art, at the time the claimed invention was made, to modify the method of Igelmund et al by replacing the mouse ES cells to human cells disclosed by Itskovitz Eldor in order to determine the electrical interaction between cardiomyocytes and beating activity of human cardiomyocytes. The Examiner's rejection is respectfully traversed. Claim 176 and dependents therefrom has now been amended. Claims 188-195 have now been cancelled.

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Applicants wish to point out that presently claimed invention (claim 176 and claims dependent therefrom) is not anticipated nor rendered obvious for the above reasons set forth above and as such withdrawal of this rejection is respectfully submitted. Therefore, it is the Applicants strong view that either alone or in combination with Igelmund, et al. Itskovitz Eldor do not anticipate or render obvious the present invention as now claimed.

In view of the above amendments and remarks it is respectfully submitted that claims 176-181, 186 and 196-199 are now in condition for allowance. A prompt notice of allowance is respectfully and earnestly solicited.

Respectfully submitted,

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Date: April 19, 2007

Enclosures:
Petition for Extension of Time (3 Months)
Additional Claims Fee
Declarations of Michal Amit (also CV); Lior Gepstein; Joseph Itskovitz-Eldor; and Itzhak Kehat
Reference by Olsen et al, Sizing up the heart: development redux in disease. Genes and Development 17:1937-1956, 2003